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Dealing with Environmental Issues of GM Crops in an Isolated Place We Call Home

Ania M. Wieczorek

College of Tropical Agriculture and Human Resources, Department of Tropical Plant and Soil Science, University of Hawaii at Mānoa, 3190 Maile Way, St. John 102, Honolulu, HI, 96822
Corresponding author, e-mail: ania@hawaii.edu

ABSTRACT

Island populations have a much higher risk of extinction than their mainland counterparts for a number of reasons. Particular concern has been voiced that gene flow and hybridization between agricultural crops and native plant species may exacerbate their precarious position, especially if the gene flow occurs from crops developed through recombinant DNA technologies. Horizontal gene transfer (HGT) and vertical gene transfer (VGT) are the two possible ways for gene flow and introgression to occur. VGT is more likely to facilitate gene transfer between agricultural crops and native plant species, although this too is dependent on a variety of factors. In this critical review phylogenetic tribal boundaries were used as a limit to hybridization potential. Overlap was found between agricultural crops and native species in four tribes: *Heliantheae*, *Gossypieae*, *Solaneae*, and *Phaseoleae*. In each tribe the factors which increase and decrease the likelihood of hybridization were evaluated and distribution analyses performed. In general it is concluded that hybridization potentials are low for most species (except *Gossypium tomentosum* which is known to hybridize with its cultivated relatives), however, small scale pollination studies should be performed for each tribe to quantify the risk and to better manage populations of native species.

Key words: Gene flow; Genetically engineered; Hybridization; Introgression.

INTRODUCTION

The Hawaiian archipelago is the most isolated island group in the world, separated by almost 1,600 km to other islands and nearly 4,000 km to the next major land mass. Due to this isolation, and 1-5 million years of evolution on the different islands (McDonald *et al.*, 1986), Hawai'i has become home to more than 1,000 native flowering plant species. There are 88 families and 211 genera represented by these species, of which over 90% are endemic (Sohmer and Gustafson, 1987). Rare species have a high priority in conservation programs because of their vulnerability to extinction by natural processes and human disturbances (Levin *et al.*, 1996).

Island populations have a much higher risk of extinction than mainland populations (Frankham, 1998; Smith *et al.*, 1993) due to human activities, over-exploitation, habitat destruction, and the introduction of competitive species (Reid and Miller, 1989; Levin *et al.*, 1996; Rieseberg, 1991).They are susceptible to hybridization because of their small numbers and their frequent proximity to more abundant congeners (Crawford *et al.*, 1987; Carlquist, 1974). Insular species are more prone to interbreeding because they tend to be less genetically divergent and to have weaker crossing barriers than their continental counterparts (Crawford *et al.*, 1987). Another factor that increases hybridization rates in insular populations is that they often have poorly differentiated floral architecture, which facilitates interspecific cross-pollination (Carlquist, 1974) or restricts self pollination (Fryxell, 1979). Rare insular species are also particularly vulnerable to hybridization because gametic wastage, reduced seed-set, and the production of ill-fit progeny on the one hand, and the swamping effect of gene flow on the other, are not likely to be counterbalanced by immigration from nonspecific populations (Levin *et al.*, 1996).

A key point that needs to be made regarding the central focus of this paper, potential for gene flow from agricultural crops to wild species, is that gene flow occurs widely in nature. This gene flow is independent of whether transgenes are involved (Haygood *et al.*, 2003). Pollen from agricultural crops often reaches wild plants growing nearby, and when the wild species are closely related to the crops, hybridization often ensues (Ellstrand *et al.*, 1999). In order for crop genes to be transferred to wild populations via hybridization, crop plants and their wild relatives need to occur sympatrically, overlap in flowering time, and be cross-compatible (Keeler and Turner, 1991). In short, if a crop and wild relative co-exist, the frequency of hybridization under natural conditions will strongly depend both on their relatedness and on the phenology and pattern of pollen dispersal (Lavigne *et al.*, 2002).

Due to the sensitivity of island ecosystems, and the fact that a large percentage of the endemic flora of Hawaii are federally listed as endangered species, concern has been voiced that gene flow and hybridization between agricultural crops and these native species may worsen their precarious position. Of particular concern is that gene flow occurs from agricultural products that have been modified through recombinant-DNA or biotechnological techniques to display traits such as insect or herbicide resistance, and the potential creation of "super plants" which could disperse freely into native ranges.

In this review two potential methods of gene transfer are considered, namely horizontal gene transfer (HGT) and vertical gene transfer (VGT). Which native genera overlap with agricultural crops was also determined by considering phylogenetic relationships. Following determining which native genera are

potentially at risk, the factors increase or decrease the likelihood of gene flow in an attempt to justify the potential for hybridization and gene flow are considered.

Native Plant Species vs. Cultivated Crops

As this proposal considers the potential for gene flow from agricultural crops grown in Hawaii to the native plant species, crops currently being produced in Hawaii are considered primarily. However, crops that are currently the focus of research with transgenic characteristics, and which may be considered for Hawaii in future, were also included. Crops for which permits have been acknowledged or are pending in Hawaii are: rice, wheat, corn, pineapple, soybeans, coffee, lime and tobacco. Furthermore, deregulated transgenic corn, soybeans and papaya are being, or have been, grown commercially in the State of Hawaii.

To determine which native plants are related to agricultural products that possibly fall into this category as well as to determine which native species are potentially at risk, a phylogram of plants depicting evolutionary relatedness based on recently published mostly molecular phylogenies was obtained (Angiosperm Phylogeny Group (APG), 1998). In this way the interrelationship between the flowering containing native genera of the Hawaiian Islands or agriculturally significant crops could be visualized to indicate where a potential overlap occurs. This shows the relatedness between these organisms, since cross compatibility is an important factor in determining if hybridization is likely to occur between two species.

Subsequently, the phylogenetic information was related to the agriculturally relevant crops produced in Hawai‘i (http://www.nass.usda.gov), focusing particularly on crops with known wild relatives. It is unlikely for species belonging to different

tribes to exhibit cross-compatibility (G. Carr, personal communication). In this way it was possible for us to further hone the list of native species potentially at risk. The family and tribe of each relevant agricultural product, as well as the native genera found in each tribe are cross listed (Table 1). Subsequently cross compatibility was determined between the native genera and the agricultural product (i.e. physiological crossing barriers) to determine if potential for hybridization exists between the two species. An evaluation was then made of the locations of native species relative to regions where agricultural crops are being grown (i.e. the role of spatial distance on gene flow) (Ellstrand, 2003). The relationship and potential for hybridization is discussed individually for each crop.

Table 1. Phylogenetic relationship between agriculturally significant crops and native plant genera. Tribes showing overlapping agricultural crops and native plant genera are shown in bold.

Family	Tribe	Agricultural Product	Genera containing native species
Araceae	*Colocasieae*	Taro- *Colocasia*	
Poaceae	*Oryzeae*	Rice- *Oryza*	
	Triticeae	Wheat- *Triticum*	
	Andropogoneae	Sugarcane- *Saccharum*	
	Maydeae	Maize- *Zea*	
	Agrostideae		*Agrostis, Calamagrostis, Deschampsia*
	-----		*Cenchrus*
	Andropogoneae		*Chrysopogon, Ischaemum*
	Paniceae		*Dichanthelium*
	-----		*Dissochondrus*
	Eragrosteae		*Eragrostis*
	Poeae		*Festuca, Poa, Trisetum*
	-----		*Isachne*
	Leptureae		*Lepturus*
	Paniceae		*Panicum*
Bromeliaceae	-----	Pineapple- *Ananas*	
Musaceae	-----	Banana- *Musa*	
Fabaceae	***Phaseoleae***	**Soybeans- *Glycine***	***Canavalia, Erythrina, Mucuna, Strongylodon, Vigna***
	Acacieae		*Acacia*
	Caesalpinieae		*Caesalpinia*

	Mimoseae		*Kanaloa*
	Robinieae		*Sesbania*
	Sophoreae		*Sophora*
	Vicieae		*Vicia*
Caricaceae	-----	Papaya- *Carica*	
Brassicaceae	*Brassiceae*	Rapeseed- *Brassica*	
	Lepidieae		*Lepidium*
Malvaceae	***Gossypieae***	**Cotton- *Gossypium***	***Gossypium, Kokia***
	Abutileae		*Abutilon*
	Hibisceae		*Hibiscus*
Rubiaceae	*Coffeae*	Coffee- *Coffea*	
	Bobeinae		*Bobea*
	Canthieae		*Canthium*
	-----		*Coprosma*
	Gardenieae		*Gardenia*
	Hedyotideae		*Hedyotis*
	Morindeae		*Morinda*
	Spermacoceae		*Nertera*
	Psychotrieae		*Psychotria*
Solanaceae	-----	Tobacco- *Nicotiana*	
	-----	**Potato- *Solanum***	***Solanum***
	Lycieae		*Lycium*
	-----		*Nothocestrum*
Asteraceae	***Heliantheae***	**Sunflower- *Helianthus***	***Argyroxiphium**, Bidens, Dubautia**, Lipochaeta**, Wilkesia****
	Artemisieae		*Artemisia*
	Vernonieae		*Hesperomannia*
	-----		*Lagenifera**
	-----		*Remya**
	-----		*Tetramolopium**

* Genera currently in the family: Compositae. Some will be considered as *Heliantheae* for reasons based on morphology, historical classification, and their potential for intergeneric hybridization.
----- No tribal classification.

Sunflower (*Helianthus annuus* L.)

Native Genera: Argyroxiphium (DC)- {Hawaiian: *ʻĀhinahina*}, Silversword, ʻEke silversword, Kaʻū silversword; Bidens (L.)- {Hawaiian: *Koʻokoʻolau, koʻolau*}, Spanish needle, beggartick; Dubautia (Gaud.)- {Hawaiian: *Naʻenaʻe, kūpaoa*}; Lipochaeta (DC)- {Hawaiian: *Nehe*}; Wilkesia (A. Gray)- {Hawaiian: *Iliau*}.

Factors affecting gene flow from sunflower to native genera

Cultivated sunflower has been shown to be cross-compatible with wild *H. annuus* and other species in the genus, which are largely endemic to western North America (Rogers *et al.*, 1982).Hand pollinations between wild sunflowers and cultivated sunflowers have resulted in fertile hybrids (Burke *et al.*, 2002). Spontaneous hybridization at substantial rates and over distances of up to 1,000 m from the crop has been detected. 10% (299 of 3,000) of seeds tested for hybridization with wild *H. annuus* planted at various distances were hybrid individuals (Arias and Rieseberg, 1994). In some crosses, the fertility of the hybrid plants did not vary significantly from the purely wild plants (Snow *et al.*, 1998). Introgression of crop alleles into wild *H. annuus* is likely to be impeded rather than prevented by the lower fitness of the hybrids (Ellstrand *et al.*, 1999).

Both cultivated and wild sunflowers are pollinated primarily by honey bees. Bumble bees, wild bees, and other insects may also be pollinators (http://www.iia.msu.edu). Similarly, *A. sandwicense* is pollinated by a bee belonging to the genus *Nesoprosopis* spp., whereas *Bidens* spp. is visited by bees, moths, and butterflies for pollination. Sunflower pollen is spiny, and thus well adapted to being transported by insects and therefore may be transported over great distances. Since the pollen is relatively heavy, wind pollination is negligible (Fick, 1978). A shared pollinator may effect intergeneric hybridization, even at great distances.

Regulations implemented for transgenic plants by the United States Department of Agriculture - Animal and Plant Health Inspection Services (USDA-APHIS), requires a separation distance of ~800 m between hybrid (or non-hybrid) sunflowers and a source of pollen which has the potential to hybridize with these, as well as a distance

of ~1,600 m between oil and non-oil sunflower types and between either type and other volunteers or wild types (http://www.aphis.usda.gov).

In order to evaluate the native species that fall into areas where *Helianthus* pollen may be found, a distribution analysis was performed for the islands of Kaua'i, O'ahu, Maui, and Hawaii. It was found that *Argyroxiphium* and *Wilkesia* do not naturally occur in any regions where viable sunflower pollen is likely to be found at any significant levels, however, many populations of *Bidens, Dubautia*, and *Lipochaeta* spp. fall directly within the agricultural areas, or close enough to the borders, to potentially be at risk.

Although cultivated sunflower generally matures more rapidly than wild sunflowers, they often exhibit some degree of phenological overlap (Burke *et al.*, 2002). Due to the climatic conditions in the Hawaiian Islands, sowing of crops is not generally restricted by seasonal factors. Consequently, *Helianthus* may potentially be in flower at any time of the year, and the native plant genera have variable flowering times which may coincide with *Helianthus* flowering

Cotton (*Gossypium barbadense* L.; *G. hirsutum* L.)

Native Genera: Gossypium (Nutt. ex Seem.)- {Hawaiian: *Ma'o, huluhulu*}; Kokia (Lewton)- {Hawaiian: *Koki'o, hau hele 'ula*}.

Factors affecting gene flow from cotton to native genera

Fryxell (1979) suggested, on the basis of morphology, that *G. tomentosum* is at risk of extinction as a result of hybridization with *G. hirsutum.* The ability of these species to hybridize has been demonstrated in breeding programs

(http://www.aphis.usda.gov). The two species interbreed freely as they are closely related, and swamping of the genotype increases the risk of extinction.

G. tomentosum is reported to be cytogenetically similar to and equidistant from *G. barbadense* and *G. hirsutum* (DeJoode and Wendel, 1992). Ironically, although all the information suggests a threat to Hawaiian cotton from *G. hirsutum*, the only reported natural hybridization was between *G. tomentosum* and *G. barbadense* in western O'ahu (Stephens, 1964). Intermediacy was found in the hybrid with respect to pubescence, leaf nectarines, corolla colors, and petal spots. A population of hybrids (*G. barbadense* x *G. tomentosum*) is known from the Nanakuli area (western O'ahu) (C. Morden, personal communication).

Cultivated cotton is a facultative self-pollinator and an opportunistic out-crosser when insect pollinators are present (Oosterhuis and Jernstedt, 1999; Free, 1993). Cotton pollen is relatively large and heavy, and not easily dispersed by wind (Fryxell, 1979; Jenkins, 1992). Insects, especially honey bees (*Apis mellifera* L.), are considered the major vectors of cotton pollen (Mamood *et al.*, 1990). The pollen remains viable for approximately 12 hours after release (Govila and Rao, 1969). Gene flow from transgenic to non-transgenic cotton has been observed in several cases through pollen dispersal studies. The transgene was found in the progeny seed at a significant value in the first border row, but this value dropped below 1% within 7 m. A low level of outcrossing (<1%) could be measured sporadically to about 25 m (Umbeck *et al.*, 1991). In a similar Australian study, the outcrossing distance was even shorter (Llewellyn and Fitt, 1996).

The USDA-APHIS regulation previously required a minimum isolation of ~30 m, if the contaminating cotton source differs by easily observable morphological characteristics from the field to be inspected. The isolation distance was ~200 m for Foundation, Registered, and Certified seed classes. A recent amendment to the regulation stipulates that, "a 12 m-wide perimeter of non-transgenic cotton could surround the transgenic plants to act as pollen sink for insect pollinators." The perimeter cotton would be disposed of by harvesting, disking and monitoring." (http://www.aphis.usda.gov).

Though *Gossypium* spp. are normally fully self-compatible, *G. tomentosum* has developed a very long style through evolution. This style elevates the stigmatic surface above the androecium making spontaneous self-pollination an extremely rare event. The result is that without a pollinator, *G. tomentosum* fails to set seed. Other evolutionary adaptations of *G. tomentosum* include: the absence of a dark petal spot, the presence of a distinctive glossy yellow petal color, the ability of the flowers to remain open during the night, and a weakly developed floral odor. These adaptations suggest that *G. tomentosum* is pollinated by moths (Fryxell, 1979).

K. kauaiensis is the only species in the *Kokia* genus that survives in its natural habitat, although this too is severely threatened. The other species in the genus survive as cultivated park or street specimens and are therefore unlikely to come into contact with a hybridizing pollen source.

In this distribution analysis, it was that found many populations of both *G. tomentosum* and *Kokia* spp. which fall into agricultural areas on the main islands of Hawai'i. Though their distribution is not extensive, the knowledge of the

hybridization potential (*G. tomentosum* x *G. barbadense*) and their low population numbers place *G. tomentosum* and *Kokia* spp. in a very precarious position.

Potato (*Solanum tuberosum* L.)

Native Genera: *Solanum* (L.)

Factors affecting gene flow from potato to native genera

The genus *Solanum* exhibits allopolyploidy amongst many of its species. *S. tuberosum* ssp. *tuberosum* L., an allohexaploid (2n = 72) is grown worldwide. *S. tuberosum* ssp. *andigena* Juz. *et* Bu., an allotetraploid (2n = 48), is cultivated mainly in Mexico and Central and South America (Hanneman, 1994). The Hawaiian *Solanum* species are allodiploids (2n = 24) (Wagner *et al.*, 1999).

Potato can self pollinate or cross pollinate (McPartlan and Dale, 1994). Early spring potatoes may be harvested before flowering, however, for maximum yields harvesting does usually not occur before the vines have begun to die (Marr, 1992). Potato flowers are unattractive to honeybees as they produce no nectar, and bumblebees (*Bombus* spp.) have been observed as the main pollinators of potatoes (McPartlan and Dale, 1994). Another important pollinator of *S. tuberosum* is the potato beetle (*Meligethes aeneus*) (Skogsmyr, 1994). Many potato cultivars are fertile while other cultivars are fully or partially sexually sterile. This sterility may limit opportunities for hybridization (Ellstrand *et al.*, 1996).

Most tuber-bearing *Solanum* species are cross compatible; although *S. tuberosum* is strongly incompatible with tuber-bearing *Solanum* species in the United States and Canada (Love, 1994). No evidence has been reported of naturally occurring intergeneric hybrids with *Solanum* as one parent.

Isolation barriers are strong between *S. tuberosum* and the thousands of non-tuber-bearing *Solanum* species (Ellstrand, 2003). McPartlan and Dale (1994), performed a pollination study in England to determine whether *S. tuberosum*_will spontaneously mate with two non-tuber-bearing species, *S. nigrum* and *S. dulcamara*. No hybridization was detected between the cultivated and these non-tuber-bearing *Solanum* species. No studies regarding the hybridization between *S. tuberosum* and the native Hawaiian *Solanum* species have been published.

Pollen dispersal in potatoes is dependant on the potato cultivar fertility, the type of pollinator, and the prevailing weather conditions (Treu and Emberlin, 2000). McPartlan and Dale (1994), found gene flow between transgenic and non-transgenic "Desiree" plants to be 2% at 3 m distance from the pollen source, and 0.017% at 10 m. The USDA-APHIS, recommends an isolating distance of ~9 m between male-fertile transgenic potato plants and non-transgenic varieties to prevent gene flow from occurring (http://www.aphis.usda.gov).

S. tuberosum does not represent a major agricultural crop in Hawaii (http://www.nass.usda.gov). The concern is based on subsistence cultivation of potatoes by homeowners. This cultivation could potentially aid pollen dispersal into areas where wild *Solanum* species may be encountered. A distribution analysis was performed between the major agricultural areas and the reported *Solanum* populations in order to provide an overview of populations that could potentially be at risk. Areas where both native and cultivated *Solanum* species may overlap and where potential exists for hybridization on all of the major islands were identified.

Soybeans (*Glycine max* L.)

Native Genera: *Canavalia* (Adans.) *Erythrina* (L.)- *Mucuna* (Adans.)- *Vigna* (Savi)-

Factors affecting gene flow from soyabeans to native genera

Cultivated soybeans (*G. max*) represent one of the few known members of the tribe: *Phaseoleae* that show stabilized polyploidy. Other members in this tribe generally have diploid genomes with 2n = 20 or 22 (e.g. *Canavalia* spp., *Mucuna* spp., *Vigna* spp.), with the exception of *Erythrina* spp. (2n = 42) which is apparently an ancient allotetraploid (Lackey, 1980). Interspecific hybridization resulting in wild plants morphologically intermediate to *G. max* and *G. soja* has been shown to spontaneously occur near Asian fields when *G. soja* is present (Abe *et al.*, 1999). The resulting stabile hybrid (*G. gracilis*) is intermediate to and interfertile with both *G. max* and *G. soja* (Ellstrand, 2003; Nakayama and Yamaguchi, 2002). No report was found of a natural intergeneric hybridization with *G. max* as the pollen parent.

Since the cultivated soybean is a predominantly self pollinated species (Nakayama and Yamaguchi, 2002), pollen drift is unlikely to play a major role in hybridization with wild relatives. Cross-pollination rates ranging from 0.03% to 0.44% at 1 m from the pollen source were observed in a three-year study in Arkansas (Caviness, 1966). At distances of more than ~4.5 m from the pollen source, natural cross-pollination was rare and did not vary greatly. However, during this study it was also shown that it is not possible to completely eliminate cross-pollination at distances greater than 4.5 m (Caviness, 1966). Furthermore, pollination normally occurs prior to the flower opening (Boerma and Moradshahi, 1975), thereby further reducing the potential for cross-pollination. Nevertheless studies have shown that soybeans may be pollinated by insect vectors, the most likely being the honey bee (*A. mellifera*). Bumble bees and pollen feeding thrips may also act as pollinators (Nakayama and Yamaguchi,

2002). Pollination studies performed investigated the pollen movement within and between rows to male-sterile soybeans suggest that a distance of approximately 18 m would be sufficient to restrict pollen contamination below a significant level (Boerma and Moradshahi, 1975). The highest rate of cross-pollination recently reported was when white flowered and purple flowered *G. max* cultivars were alternately grown within a row (15.2 cm apart). The dominance of purple flower color over the white color was used to identify cross-pollination and the rates ranged from 0.65 to 6.32%, with an average of 1.8%. The rates of cross-pollination was significantly less (0.03 to 0.05%), when the white flower color cultivar was grown at a distance greater than 5 m from the purple color cultivar in natural crosses between rows. These results are comparable to values previously reported (Ray *et al.*, 2003).

Regulations concerning isolation distances between transgenic soybean varieties and non-transgenic varieties, implemented by the USDA-APHIS, require a distance adequate to prevent mechanical mixture (http://www.aphis.usda.gov).

Since no information was found concerning the possibility of intergeneric hybridization, it was assumed that this represents a potential risk to the native Hawaiian plant species, until shown otherwise. For this reason a distribution analysis of the five native Fabaceae species in relation to the major agricultural areas was performed. In general overlapping areas where both native and cultivated plants may be present are on all the major islands, and though cross pollination does not occur over vast distances, some species may still be at risk.

FINAL CONCLUSIONS AND RECOMMENDATIONS

In this study, four agricultural crops, namely sunflowers (*H. annuus*); cotton (*G. barbadense*, *G. hirsutum*); potatoes (*S. tuberosum*); and soybeans (*G. max*) were considered for their potential to hybridize with native relatives in the Hawaiian archipelago. Tribal boundaries were considered to be a primary limit to hybridization potential since, at greater intergeneric distances, hybridization has not been documented for any taxa. Genotypic relationship is not the only factor that determines whether gene flow is likely to occur. Biological barriers, physical barriers and environmental factors all play a role in limiting or enhancing gene flow by vertical gene transfer (Grant, 1975; Ellstrand *et al.*, 1999; Wolf *et al.*, 2001).

Insular species are at greater risk of extinction than mainland populations (Levin *et al.*, 1996; Wolf *et al.*, 2001), and hence hybridization is a potential threat. Various factors contribute to a higher extinction rate of insular species, including inbreeding depression, a loss of genetic variation through genetic assimilation, or genetic adaptation to island conditions. Insular species are coming into contact with non-native species at higher frequencies due to human processes that increases the potential for gene flow between these groups (Levin *et al.*, 1996).

The USDA-APHIS suggests isolation distances between transgenic and non-transgenic plant species, to minimize the potential for hybridization to occur. It is recommended that the USDA-APHIS isolation distances be strictly employed to separate any agricultural crop from native Hawaiian plant relatives. Further, laboratory pollination studies should be performed for the crops and native genera that were considered in this report to confirm whether cross-pollination is possible among these species.

It is crucial to determine whether hybridization may occur under natural conditions, while hand pollination should provide sufficient evidence to determine if hybrid progeny are produced. Any hybrid progeny should be evaluated for fertility. Although hybridization seems unlikely, quantifying this risk with experimental data is important.

In addition to the agricultural products discussed in this study, many native plant species have relatives that have become naturalized in the Hawaiian Islands. One such example is the genus *Solanum.* It seems unlikely for *S. tuberosum* to hybridize with any of the four native members of the *Solanum* genus, however, of the eleven naturalized non-tuber-bearing *Solanum* species that exist in the Hawaiian archipelago all may have a higher likelihood to hybridize to the native species. Often the naturalized relatives share habitats that bring them into close proximity of indigenous species. It is recommended that hybridization and introgression between these groups be further investigated.

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